

# Assessing Postpartum Reproductive Health via Endometrial Cytology and Body Temperature in Mehsana Buffaloes

## ABSTRACT

The aim of this study was to investigate the effect of body temperature and endometrial cytology on postpartum reproductive health in Mehsana buffaloes. A total of twenty-six Mehsana buffaloes were used in this study. Per-rectal and ultrasonography examinations were used to observe the postpartum reproductive function of these buffaloes and on day 60 post natural service, the pregnancy was confirmed. Based on fertile estrus, buffaloes were retrospectively divided into two groups: Group I (buffaloes conceiving before 90 days) and Group II (buffaloes conceiving after 90 days). One week before parturition and four weeks after it, the rectal temperature was taken. The cytobrush technique was used to obtain endometrial samples for cytological tests on the 21<sup>st</sup> and 30<sup>th</sup> day postpartum. During the final week of pregnancy and the first, second, third, and fourth weeks' post-partum, the mean rectal temperature ( $^{\circ}$  F) varied from  $101.7 \pm 0.07$  to  $102.0 \pm 0.06$  in Group-I and  $101.6 \pm 0.07$  to  $102.1 \pm 0.05$  in Group-II. During the final week pre-partum and the first week post-partum, the mean rectal temperature ( $^{\circ}$  F) was shown to be significantly ( $p < 0.05$ ) lower than it was during the second, third, and fourth weeks post-partum among the groups. On the 21<sup>st</sup> and 30<sup>th</sup> day postpartum during endometrial cytology, the percentage of neutrophils was  $14.31 \pm 1.56$  and  $12.23 \pm 0.93$  in Group I and  $17.92 \pm 1.51$  and  $15.08 \pm 0.73$  in Group II, respectively. On the 30<sup>th</sup> day, there was a significant difference ( $p < 0.05$ ) between the groups in Group II that had a greater mean percentage of neutrophils. When endometrial cytology was performed on buffaloes at various postpartum days, the mean percentage of lymphocytes, endometrial epithelial cells and large vacuolated epithelial cells revealed non-significant changes. On the 30<sup>th</sup> day, it was discovered that the prevalence of subclinical endometritis in Group I and Group II buffaloes was 7.69 and 23.07 percent, respectively. The postpartum first behavioural estrus and first fertile estrus occurred at  $62.00 \pm 4.34$  and  $65.31 \pm 4.45$  days in Group I and  $77.46 \pm 11.12$  and  $130.08 \pm 9.26$  days in Group II buffaloes, respectively. Although, the average days for first behavioural estrus did not differ significantly between the groups the fertile estrus occurred significantly ( $P < 0.05$ ) delayed in Group II buffaloes.

**Keywords:** Cytobrush technique, endometrial cytology, Mehsana buffaloes, polymorphonuclear cells

## INTRODUCTION

Buffaloes play a significant role in India's agricultural economy and are mostly used as a source of milk and meat in tropical and subtropical regions. After calving, the health of the uterus has a significant impact on the reproductive success of dairy cows. Reproductive performance is negatively impacted by postpartum

endometritis because it lowers the rate of conception and increases services per conception (Fourichon *et al.*, 2000). Compared to cows, buffalo had a significantly higher prevalence rate of uterine infection (Moghaddam & Mamoei, 2004). There are clinical and subclinical subtypes of endometritis. A uterine discharge that is purulent or mucopurulent and appears 21 or 26 days postpartum is considered clinical endometritis. When uterine cytology samples taken between 21 and 33 days postpartum show more than 18% polymorphonuclear (PMNs) cells or when samples taken between 34 and 47 days postpartum show more than 10% PMNs, it is considered subclinical endometritis. Although subclinical endometritis does not cause uterine discharge, the disease is nonetheless thought to be severe enough to affect a cow's ability to reproduce (Sheldon *et al.*, 2006). Uterine cytology is a new endometritis diagnosis technique that has been employed recently in bovine gynaecology mostly to identify subclinical endometritis in clinically healthy cows. The primary inflammatory cell type observed in intrauterine fluid accumulations is polymorphonuclear cells (PMNs). Research has demonstrated that the relative number of PMNs can predict the reproductive performance of postpartum cows. (Gilbert *et al.*, 2005; Kasimanickam *et al.*, 2004). It has been proposed that the cytobrush technique is a more consistent and dependable way to extract inflammatory cells (Kasimanickam *et al.*, 2005). Dairy producers and veterinary professionals have embraced the methodology for tracking postpartum cows' daily rectal temperatures since it provides an objective response that can be utilized to assess health. The majority of research in the literature now under publication focuses on the connection between puerperal metritis and rectal temperature; however, there is little data connecting postpartum endometrial cytology, subclinical endometritis and rectal temperature in periparturients. While postpartum buffalo rectal temperature is frequently used as a monitoring tool, nothing is known about the use or importance of rectal temperature and postpartum endometrial cytology investigation as diagnostic techniques for sub-clinical endometritis in buffalo. This being the case, the suggested study on rectal temperature and endometrial cytological examination during the periparturient phase and the occurrence of the first post-partum estrous with behavioural indications in Mehsana buffaloes was carried out considering these facts.

## **MATERIALS AND METHODS**

### **Animals**

A total of 26 Mehsana buffaloes, irrespective of parity and body weight with a history of normal calving kept at Livestock Research Station, SDAU, Sardarkrushinagar, Gujarat, were used for the present study. According to the farm's regular feeding schedule, all of the buffaloes were kept in clean well-ventilated sheds and fed fresh fodder, hay and compounded concentrate. Throughout the study period, the zone's average lowest and maximum ambient

temperatures as well as relative humidity ranged from 17.95 °C to 32.25 °C and 48.5% to 73%, respectively.

### **Body Temperature**

The rectal temperature of these buffaloes was recorded in the morning hours from one week prior to parturition to four weeks after parturition with a digital thermometer.

### **Uterine Sample Collection**

Endometrial samples for cytological examinations were collected on the 21<sup>st</sup> and 30<sup>th</sup> days postpartum by using a cytobrush modified for use in large animals. The normal cytobrush was attached to a stainless steel catheter for passage through the cervix. The buffaloes were restrained with their tail secured to one side and the vulva and perianal regions were washed and cleaned. The vulva was dried with a cotton swab prior to passing the instrument through the vagina to the external cervical os and the instrument was advanced through the cervix into the uterus. The cytobrush was exposed against the endometrium by pushing the inner rod of the catheter forward. Endometrial cytology samples were collected by rotating the cytobrush in a clockwise direction while in contact with the uterine wall. The cytobrush was retracted into the stainless steel tube prior to removal from the uterus. The stainless steel device was cleaned with alcohol to avoid contamination between uses.

### **Endometrial Cytology**

Smears for cytological examination were prepared by rolling the cytobrush on to a clean glass microscope slide and fixing them with methanol for 1 minute. Smears were then stained with field stain (eosin for 45 seconds and methylene blue for 45 seconds) and air-dried. Cytological assessment was performed by counting a total of 100 cells at 400x and 1000x magnification to determine the percentage (%) of neutrophils (PMNs). Initially, the whole slide was assessed and a representative area was selected to determine the neutrophils, lymphocytes, endometrial epithelial cell and large vacuolated epithelial cells. Slides for cytological examination were assessed twice to estimate polymorph nuclear leukocytes/neutrophils per cent (%). Buffaloes with >18 % PMNs in the uterine samples were regarded as affected by subclinical endometritis with no gross signs of endometritis as per Sheldon *et al.*, (2006)

## Statistical Analysis

The collected data were subjected to statistical analysis using the SPSS software, version 18. Analysis of Variance (ANOVA) was conducted to check for significant differences between the means of rectal temperature as well as endometrial cytology between the groups of animals. As per usual statistical technique, the data gathered from research work were statistically analysed using the "t-test." (Snedecor and Cochran, 1994). *p*-value less than .05 was considered as significant.

## RESULTS AND DISCUSSIONS

### Rectal Temperature

The buffaloes in Group I and Group II had average rectal temperatures ( $^{\circ}$  F) ranging from  $101.7 \pm 0.07$  to  $102.0 \pm 0.04$   $^{\circ}$  F and  $101.6 \pm 0.07$  to  $102.0 \pm 0.04$   $^{\circ}$  F, respectively (Table 1 and Figure 1). The study observed rectal temperature ranges that are in good agreement with Chandra Bhan (2012) observations on cattle. In comparison to the second, third and fourth weeks postpartum within the groups, the mean rectal temperature ( $^{\circ}$  F) was observed to be considerably ( $p < 0.05$ ) lower during the last week of prepartum and the first week postpartum. Whereas, the difference observed was non-significant between the groups at all other periods and among the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> weeks postpartum within both groups (Table 1). Vickers *et al.* (2010) showed higher rectal temperatures in healthy cows compared to the current findings. On the other hand, Wagner *et al.* (2008) suggested that tissue damage related to calving trauma could be the cause of an elevated rectal temperature in a healthy cow. According to studies by Suthar *et al.* (2012) and Burfeind *et al.* (2014), ambient temperature affects body temperature; primiparous cows had a body temperature that was  $0.2^{\circ}$ C higher than multiparous cows.

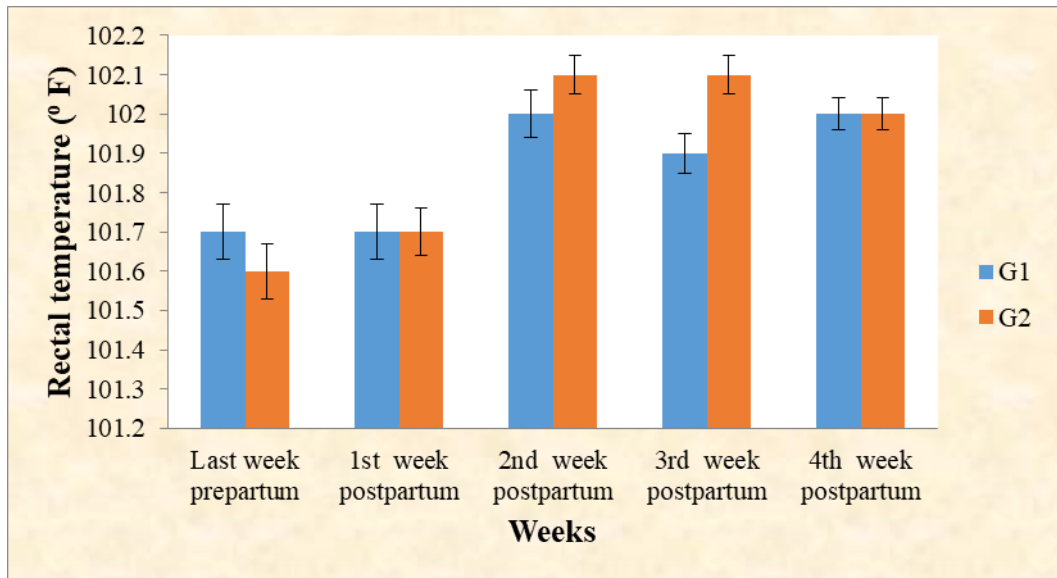
### Endometrial Cytology

#### Neutrophils

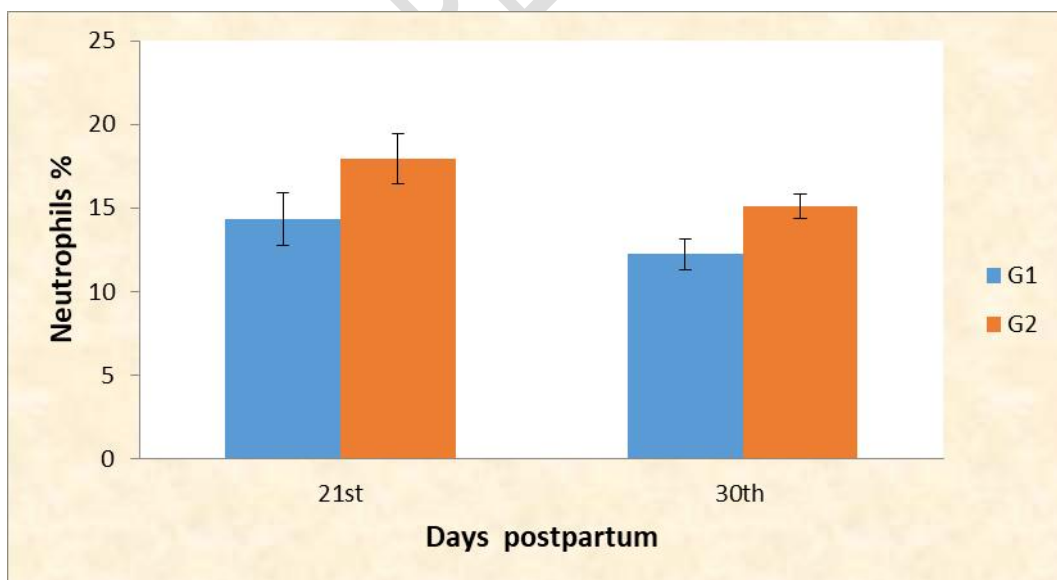
Table 2 and Figure 2 show that the mean percentage of neutrophils seen during endometrial cytology in buffaloes at the 21<sup>st</sup> and 30<sup>th</sup> day postpartum were  $14.31 \pm 1.56$  and  $12.23 \pm 0.93$  in Group I and  $17.92 \pm 1.51$  and  $15.08 \pm 0.73$  in Group II, respectively. A non-significant decline in per cent neutrophils was observed at different days postpartum within the groups of buffaloes at different periods of endometrial cytology. Comparable outcomes were seen using cytobrush techniques in cows at days 21 and 28 postpartum by Saut *et al.* (2013) and at day 26 postpartum by Brodzki *et al.* (2014).

On day 21, there was no statistically significant difference in the mean percentage of neutrophils in the buffaloes belonging to Group II and Group I. On day 30, a significant difference ( $p < 0.05$ ) was noted between the groups, with Group II exhibiting a greater mean percentage of neutrophils in comparison to Group I. The current study's results are consistent with those of McDougall *et al.* (2011), who found that cows in the highest quartile for PMNs percent at days 28 and 42

experienced a lower pregnancy rate and a longer time to conception than cows in the lower PMNs percent categories. According to Nibret Moges. (2015), following sampling, the cows that conceived during the first artificial insemination had fewer neutrophils overall. Nonetheless, Ahmadi *et al.* (2006) found no statistically significant variation in the percentage of cells in cows that were conceived through one or multiple artificial inseminations.



**Figure 1 Rectal temperature ( $^{\circ}$  F, Mean  $\pm$  SE) at different weeks in different groups of postpartum Mehsana buffaloes.**



**Figure 2 Per cent neutrophil (Mean  $\pm$  SE) count during endometrial cytology in different groups of postpartum Mehsana buffaloes.**

**Lymphocytes**

In Group-I, the mean percentage of lymphocytes detected during endometrial cytology was  $0.31 \pm 0.13$  and  $0.23 \pm 0.12$ , while in Group-II, it was  $0.38 \pm 0.14$  and  $0.23 \pm 0.12$  (Table 2). At various postpartum days, both within and between groups, there was a non-significant decrease in the percentage of lymphocytes. These research results were in good agreement with the findings in cows (Oruc *et al.*, 2015) and buffaloes (Selvaraj *et al.*, 2014). Variations in the lymphocytic image may result from differences in the species, cytology site, cytological methods, and reproductive phase.

### **Endometrial Epithelial Cells (EPC) and Large Vacuolated Epithelial Cells (LVEC)**

The mean percentage of endometrial epithelial cells observed during endometrial cytology in buffaloes at 21<sup>st</sup> and 30<sup>th</sup> day postpartum were  $80.23 \pm 2.49$  and  $84.69 \pm 1.97$  in Group I and  $81.15 \pm 1.84$  and  $83.85 \pm 1.04$  in Group II, respectively and The mean percentage of large vacuolated epithelial cells observed during endometrial cytology in buffaloes at 21<sup>st</sup> and 30<sup>th</sup> day postpartum were  $9.77 \pm 0.56$  and  $10.77 \pm 0.71$  in Group I and  $9.85 \pm 0.54$  and  $10.54 \pm 0.45$  in Group-II, respectively (Table 2). These findings corroborated well with the findings in cows at 25 to 30 days post-parturition (Saut *et al.*, 2013). In comparison to these, higher per cent of endometrial epithelial cells have been reported in buffaloes (Selvaraj *et al.*, 2014) and in ewes and dairy cows using cervical and uterine cytology during estrus phase (Benbia *et al.*, 2013). Whereas, lower values have been reported in cow using cytobrush at luteal and follicular phases of estrus cycle (Brodzki *et al.*, 2015 and Brodzki *et al.*, 2014<sup>b</sup>) and in dairy cows in cervical and uterine cytology (Yavari *et al.*, 2009).

However, the difference reported by various authors for EPC and LVEC was non-significant. Similarly, a non-significant increase in the per cent EPC and LVEC at different days postpartum within and between the groups was also observed in these studies.

### **Prevalence of Subclinical Endometritis**

**Table 1 Prevalence of subclinical endometritis at different days in different groups of Mehsana buffaloes.**

		Groups

Days of Postpartum	Neutrophils Counts (%)	G1 (n=13)		G2 (n=13)	
		Frequency	%	Frequency	%
21 <sup>st</sup>	< 18	11	84.61	9	69.23
	> 18	2	15.38	4	30.76
30 <sup>th</sup>	< 18	12	92.30	10	76.92
	> 18	1	7.69	3	23.07

Cytological smears are evaluated for their proportion of polymorph nuclear cells (PMNs) in a sample. An increased proportion of PMNs is prognostic for impaired subsequent reproductive performance (Kasimanickam *et al.*, 2004). In these studies, we adopted the criteria as per Kasimanickam *et al.* (2004) and Sheldon *et al.* (2006) who considered cows to suffer from subclinical endometritis if the PMNs exceeded 18% between 20<sup>th</sup> and 33<sup>rd</sup> days postpartum. Results show that the prevalence of subclinical endometritis at 21<sup>st</sup> day postpartum was found to be 15.38 and 30.76 per cent and at 30<sup>th</sup> day postpartum, it was 7.69 and 23.07 per cent in Group I and Group II, respectively (Table 3). These findings are in accordance with reports in buffaloes (Senosy and Hussein, 2013), in cows (Zobel, 2013 and Nibret Moges, 2015). Whereas, Saut *et al.* (2013) have reported a higher prevalence in clinically healthy crossbred dairy cows with normal delivery at days 21 and 28 postpartum by cytobrush technique. Similarly, a higher prevalence was observed on the 21<sup>st</sup> and 30<sup>th</sup> day postpartum in the group II buffaloes that were conceived after 90 days. Thus, the present study proved that the cytobrush technique can be reliably used to obtain endometrial samples as well as to know the prevalence of subclinical endometritis which is in accordance with the findings of Kasimanickam *et al.*, (2005).

### First Behavioral Estrus and Fertile Estrus

**Table 2 First behavioral and fertile estrus (days, Mean  $\pm$  SE) in postpartum Mehsana buffalo**

Estrus Parameter (Days)	Groups		't' value
	G1 (n=13)	G2 (n=13)	

<b>First behavioral estrus</b>	62.00±4.34	77.46±11.12	1.23 <sup>NS</sup>
<b>Fertile estrus</b>	65.31±4.45	130.08±9.26	6.30 <sup>**</sup>

NS= Non-significant, \*\* = Significance at 5% and 1% level.

In Group I buffaloes, the mean postpartum first behavioural estrus happened at 62.00 ± 4.34 days, while in Group II buffaloes, it happened at 77.46 ± 11.12 days (Table 4). The findings in buffaloes by Khasatiya *et al.* (2006) and Kalasariya *et al.* (2017) are in good agreement with these findings. Whereas, Rijasnaz *et al.* (2012) have reported that buffaloes exhibit relatively higher and earlier days of first behavioural estrous. In Group II buffaloes, fertile estrus was seen to occur 130.08±9.26 days later than in Group I buffaloes, with a significant difference (P<0.05). These results are well supported by the reports on crossbred cows and buffaloes with subclinical endometritis compared to those without the condition had significantly (P<0.05) lower first service conceptions and required more services for conceptions (Sinha *et al.*, 2002) and Jan Mustafa *et al.* (2019). Therefore, the different management techniques, nutritional status and subclinical uterine infection could contribute to the observed delayed fertile estrus.

**Table 3 Rectal temperature ( $^{\circ}$  F, Mean  $\pm$  SE) at different weeks in different groups of postpartum Mehsana buffaloes**

Weeks	Groups		Overall mean
	G1 (n=13)	G2 (n=13)	
Last week pre-partum	101.7 $\pm$ 0.07 <sup>a</sup> <sub>p</sub>	101.6 $\pm$ 0.07 <sup>a</sup> <sub>p</sub>	101.6 $\pm$ 0.07
1 <sup>st</sup> week postpartum	101.7 $\pm$ 0.07 <sup>b</sup> <sub>p</sub>	101.7 $\pm$ 0.05 <sup>b</sup> <sub>p</sub>	101.7 $\pm$ 0.06
2 <sup>nd</sup> week postpartum	102.0 $\pm$ 0.06 <sup>c</sup> <sub>q</sub>	102.1 $\pm$ 0.04 <sup>c</sup> <sub>q</sub>	102.0 $\pm$ 0.05
3 <sup>rd</sup> week postpartum	101.9 $\pm$ 0.05 <sup>d</sup> <sub>q</sub>	102.1 $\pm$ 0.05 <sup>d</sup> <sub>q</sub>	102.0 $\pm$ 0.05
4 <sup>th</sup> week postpartum			

	102.0±0.04 <sup>e</sup> <sub>q</sub>	102.0±0.04 <sup>e</sup> <sub>q</sub>	102.0±0.04
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Means with different superscripts within rows & subscripts within columns differ significantly at 5 per cent level.

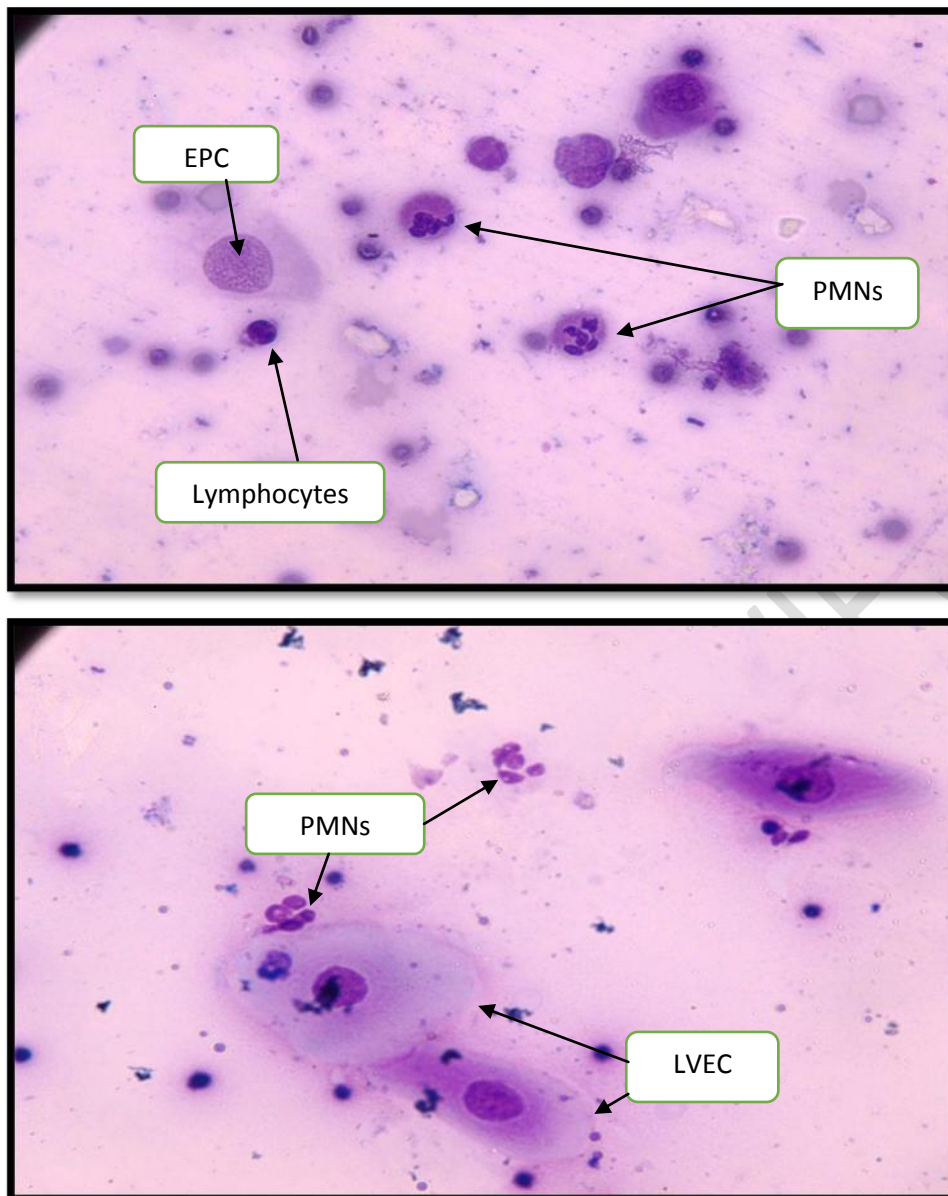
**Table 4 Different cell types (per cent, Mean ± SE) during endometrial cytology of postpartum Mehsana buffaloes**

Type of cell	Days of postpartum	Groups		Overall mean
		G1 (n=13)	G2 (n=13)	
Neutrophils	21 <sup>st</sup>	14.31±1.56 <sup>a</sup> <sub>q</sub>	17.92±1.51 <sup>a</sup> <sub>p</sub>	16.11±1.53
	30 <sup>th</sup>	12.23±0.93 <sup>a</sup> <sub>q</sub>	15.08±0.73 <sup>b</sup> <sub>p</sub>	13.65±0.83
Lymphocytes	21 <sup>st</sup>	0.31±0.13	0.38±0.14	0.34±0.13
	30 <sup>th</sup>	0.23±0.12	0.23±0.12	0.23±0.12

<b>Endometrial epithelial cells</b>	<b>21<sup>st</sup></b>	80.23±2.49	81.15±1.84	80.69±2.16
	<b>30<sup>th</sup></b>	84.69±1.97	83.85±1.04	84.26±1.50
<b>Large vacuolated epithelial cells</b>	<b>21<sup>st</sup></b>	9.77±0.56	9.85±0.54	9.81±0.55
	<b>30<sup>th</sup></b>	10.77±0.71	10.54±0.45	10.65±0.58

Means with different superscripts within rows & subscripts within columns differ significantly at 5 per cent level for each type of cell.

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**Figure 3 Different cell types during endometrial cytology at postpartum in Mehsana buffalo (Eosin & Methylene blue stain, 1000X).**

### CONCLUSIONS

The present study concluded that the rectal temperature of buffaloes that became pregnant beyond 90 days was noticeably higher in the second postpartum week. Buffaloes who conceived after 90 days demonstrated significantly higher mean percent neutrophils at the 30<sup>th</sup> day postpartum along with a higher prevalence of subclinical endometritis. Endometrial cytology revealed a significantly decreasing trend in the appearance of neutrophils at the 21<sup>st</sup> and 30<sup>th</sup> days postpartum. When it came to the groups' first behavioural oestrus, there was no significant difference. On the other hand, it was discovered that buffaloes that were

conceived after 90 days experienced a **significant** ( $P < 0.05$ ) delay in postpartum fertile oestrus.

Ethical approval:

All experimental procedures were conducted as per the guidelines of the Institutional Animal Ethics Committee (IAEC).

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